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Bacterial surface display: trends and progress

Stefan Ståhl and Mathias Uhlén

Heterologous surface display on Gram-negative bacteria was first described a decade ago and is now an active research area. More recently, strategies for surface display on Gram-positive bacteria have also been devised and these carry some inherent advantages. Bacterial surface display has found a range of applications in the expression of various antigenic determinants, heterologous enzymes, single-chain antibodies, polyhistidyl tags and even entire peptide libraries. This article explains the basis of bacterial surface display and discusses current uses and possible future trends of this emerging technology.

The targeting and anchoring of heterologous proteins to the outer surface of yeast[1] and mammalian cells[2] has been utilized for various applications, and bacterial surface display is becoming an increasingly important research area[3–7]. Surface display of heterologous proteins in bacteria has been employed as a tool for fundamental and applied research in microbiology, molecular biology, vaccinology and biotechnology. The first examples of heterologous surface display were reported a decade ago[8–10], when short gene fragments were inserted into the genes for the *Escherichia coli* outer membrane proteins LamB, OmpA and PhoE, and the gene fusion products were found to be accessible on the outer surface of the recombinant bacteria. Since then, not only outer membrane proteins but also lipoproteins, fimbria proteins and flagellar proteins, as well as dedicated systems with coupled translocation and surface anchoring, have been employed to achieve heterologous surface display on Gram-negative bacteria[3–6], for example *E. coli* and *Salmonella* spp. More recently, systems have been described for heterologous surface display on Gram-positive bacteria such as staphylococci[11], streptococci[12,13] and mycobacteria[14].

The most common application of bacterial surface display has been in the development of live-bacterial-vaccine delivery systems because the cell-surface display of heterologous antigenic determinants has been considered advantageous for the induction of antigen-specific antibody responses when using live recombinant cells for immunization[5,14,15]. In this review, we also discuss the use of bacterial surface display in generating whole-cell bioadsorbents for environmental purposes[16], novel microbial biocatalysts (enzymes surface displayed with retained activity on, for example, *E. coli*[17,18] and staphylococci[19]), diagnostic tools (bacteria with surface-displayed antibody fragments[20–22])

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and for the display of entire peptide libraries[23] (as an alternative to the rapidly developing phage-display technology). In addition, we describe novel techniques for the characterization and quantification of bacterial-surface-displayed proteins.

Surface display in Gram-negative bacteria

Display systems have been created for the expression of a number of heterologous proteins on the surface of Gram-negative bacteria (selected examples are presented in Table 1). Most often, foreign gene products have been fused to outer membrane proteins such as the maltoporin LamB, the phosphate-inducible porin PhoE and the outer membrane protein OmpA, and to lipoproteins such as the major lipoprotein Lpp, the TraT lipoprotein and the peptidoglycan-associated lipoprotein (PAL). Proteins from the filamentous structures present on Gram-negative bacteria have also been employed for surface-expression purposes, including fimbria proteins (such as the FimA protein[27] and the FimH adhesin[28] of type 1 fimbriae, and the F11 fimbrillin of P fimbriae[31]), the flagellar protein flagellin[26,35,36] and pili proteins (such as the PapA pilus subunit[34]).

Certain display systems are based on proteins that have specific mechanisms for translocation and surface anchoring. The lipoprotein pullulanase from *Klebsiella pneumoniae* has been employed for surface-display purposes in *E. coli*[32]. Using this system, the target protein becomes transiently anchored to the outer membrane by its N-terminal fatty acid moiety and is subsequently released into the culture medium. The β-domain of the *Neisseria gonorrhoeae* IgA protease precursor (Iga_β) and the *E. coli* AIDA-I have also been employed for surface exposure of various heterologous proteins[29,30]. When used as a C-terminal-fusion partner, the Iga_β and AIDA-I mediate attachment of hybrid proteins on the outer surface of *Salmonella*

Table 1. Examples of surface-display systems for Gram-negative bacteria with some areas of application

Display system (origin)	Host bacteria	Displayed protein	Comment	Refs
Surface display of antigenic determinants				
LamB (*E. coli*)	*E. coli*	C3 epitope of poliovirus	Positive immunolabelling on bacteria	8
PhoE (*E. coli*)	*E. coli*	VP1 of FMDV	Epitope recognized on bacteria by MAb	10
OmpA (*E. coli*/ *S. dysenteriae*)	*S. typhimurium*	Haemagglutinin of IAV	Partial protection in mice after oral immunization with live bacteria	24
TraT lipoprotein (*E. coli*)	*E. coli*	C3 epitope of poliovirus	Retained function of the TraT lipoprotein	25
Flagellin (*E. coli*)	*E. coli*	Hen-egg-lysozyme epitope	Functional flagella with accessible epitope	26
Fim A protein (*E. coli*)	*E. coli*	Epitopes from HBV, FMDV and poliovirus	Epitopes accessible at cell surface	27
Fim H adhesin (*E. coli*)	*E. coli*	PreS2 of HBV and CTB epitope	Epitopes accessible at cell surface	28
Iga_β (*N. gonorrhoeae*)	*E. coli*/ *S. typhimurium*	*Vibrio cholerae* CTB	CTB anchored at outer cell surface	29
AIDA-I (*E. coli*)	*E. coli*	*Vibrio cholerae* CTB	CTB anchored at outer cell surface	30
P fimbrillin (*E. coli*)	*E. coli*	Epitope from FMDV	Epitope recognized in fimbriae by MAb	31
Surface display of enzymes				
Pullulanase (*K. pneumoniae*)	*E. coli*	*E. coli* β-lactamase	Initial anchoring followed by slow release	32
Lpp–OmpA chimera (*E. coli*)	*E. coli*	*Cellulomonas fimi* cellulase	90% of hydrolase activity at bacterial surface	18
Surface display of antibody fragments				
PAL (*E. coli*)	*E. coli*	Chick-lysozyme-specific scFv	Surface accessibility demonstrated by IF	20
Lpp–OmpA chimera (*E. coli*)	*E. coli*	Digoxin-specific scFv	Enrichment by FACS of correct clones	21
Surface display of other proteins				
LamB/Pap pili (*E. coli*)	*E. coli*	SpA domains	IgG-binding activity at bacterial surface	33,34
Thioredoxin–flagellin (*E. coli*)	*E. coli*	Random 20-amino-acid library	MAb epitopes could be mapped by isolation of specific bacteria in a panning procedure	23
LamB (*E. coli*)	*E. coli*	Hexahistidyl peptides	Bacteria able to adsorb cadmium ions	16

Abbreviations: AIDA-I, adhesin involved in diffuse adherence; CTB, cholera toxin B subunit; FACS, fluorescence-activated cell sorting; FMDV, foot-and-mouth-disease virus; HBV, hepatitis B virus; IAV, influenza A virus; IF, immunofluorescence; Iga_β, immunoglobulin A protease precursor β; MAb, monoclonal antibody; OmpA, outer membrane protein A; PAL, peptidoglycan-associated lipoprotein; scFv, single-chain Fv antibody fragment; SpA, *S. aureus* protein A.

typhimurium and *E. coli* strains without the OmpT protease[29,30].

One particularly interesting system is based on the combined features of an Lpp–OmpA chimera[17]. The signal sequence and first nine amino acids of Lpp are fused to a region comprising three[37] or five[17,18] transmembrane regions of OmpA. This Lpp–OmpA display system has been shown to give efficient translocation and surface anchoring of the fused gene products, resulting in a high number of chimeric surface proteins present in an accessible form on *E. coli* cells[6,37].

Surface display in Gram-positive bacteria

Gram-positive bacteria have only recently been taken into consideration for bacterial surface display purposes[11,12]. A range of proteins, including antigenic determinants, heterologous enzymes and single-chain Fv (scFv) antibodies, have been targeted and anchored to the cell surface of Gram-positive bacteria (Table 2). Several Gram-positive bacterial hosts have been investigated in this context (Table 2), but until recently the research has focused on nonpathogenic staphylococci used in food-fermentation processes[47,48] (namely *Staphylococcus xylosus*[11] and *Staphylococcus carnosus*[40]), the mouth commensal *Streptococcus gordinii*[12] and attenuated mycobacteria[14]. Schneewind and co-workers finally elucidated the mechanisms of cell-surface targeting and subsequent anchoring of surface proteins on staphylococcal cells[49–52] several years after heterologous surface display had been achieved on staphylococci and streptococci[11,12]. They investigated how *Staphylococcus aureus* protein A (SpA) was sorted to the cell surface and suggested a highly plausible mechanism. The C-terminal surface-anchoring region of SpA (Fig. 1) consists of a charged repetitive region, postulated to interact with the peptidoglycan cell wall[53], followed by a region common to numerous cell-surface-bound receptors of Gram-positive bacteria containing an LPXTG motif, a hydrophobic region and a short charged tail[7,54]. It has been demonstrated that the latter tripartite region is required for cell-surface anchoring and that the cell-wall sorting is accompanied by proteolytic cleavage within the LPXTG motif, between the threonine and glycine residues, and subsequent covalent linking of the surface receptor to the cell wall[49–52]. The C-termini of numerous Gram-positive bacterial surface receptors are highly homologous[7,50,54], suggesting that this (or a similar) mechanism is utilized for their targeting to the cell surface.

The surface-display systems developed for *S. xylosus*[11] and *S. carnosus*[40] both take advantage of the cell-surface-anchoring regions of SpA, while the *S. gordinii* surface-display system[12] uses the similar C-terminal region of the M6 protein of *Streptococcus pyogenes* to achieve surface exposure of various chimeric surface proteins. While the staphylococcal systems utilize gene expression from plasmid vectors, the streptococcal system is based on incorporation of the target genes into the streptococcal chromosome by homologous recombination[11,55]. Recently, the *S. pyogenes* M6

Table 2. Gram-positive bacteria evaluated for surface-display applications

Bacteria	Display system	Displayed protein	Comments	Refs
Food-fermenting bacteria				
Staphylococcus xylosus	*S. aureus* protein A	Various antigens	Serum IgG after oral immunization of mice	11,15, 38,39
Staphylococcus carnosus	*S. aureus* protein A	Various antigens	Improved system compared with *S. xylosus*	39–41
S. xylosus/S. carnosus	*S. aureus* protein A	Anti-human-IgE scFv antibody	Whole cells able to bind antigen (human IgE)	22
Staphylococcus carnosus	*S. aureus* FnBPB	Enzymes: lipase/β-lactamase	Enzymes fully active at cell surface	19
Lactococcus lactis	*L. lactis* proteinase PrtP	Tetanus toxin fragment C	Antigen not accessible at outer cell surface	42
Commensal bacteria				
Streptococcus gordinii	*S. pyogenes* M6 protein	Various antigens	Serum IgG and mucosal IgA shown in mice	12,43–45
Sporulating bacteria				
Bacillus subtilis	*B. subtilis* CwbA	*Y. pseudotuberculosis* invasin	Immunization of mice with spores evokes antigen-specific antibodies	46
Attenuated pathogenic bacteria				
Mycobacterium bovis	*M. tuberculosis* membrane-associated lipoprotein	*Borrelia burgdorferi* OspA	Protective immunity evoked in mice by intraperitoneal immunization	14

Abbreviations: CwbA, cell-wall-bound autolysin modifier protein; FnBPB, fibronectin-binding protein B; OspA, outer surface protein A; scFv, single-chain Fv antibody fragment.

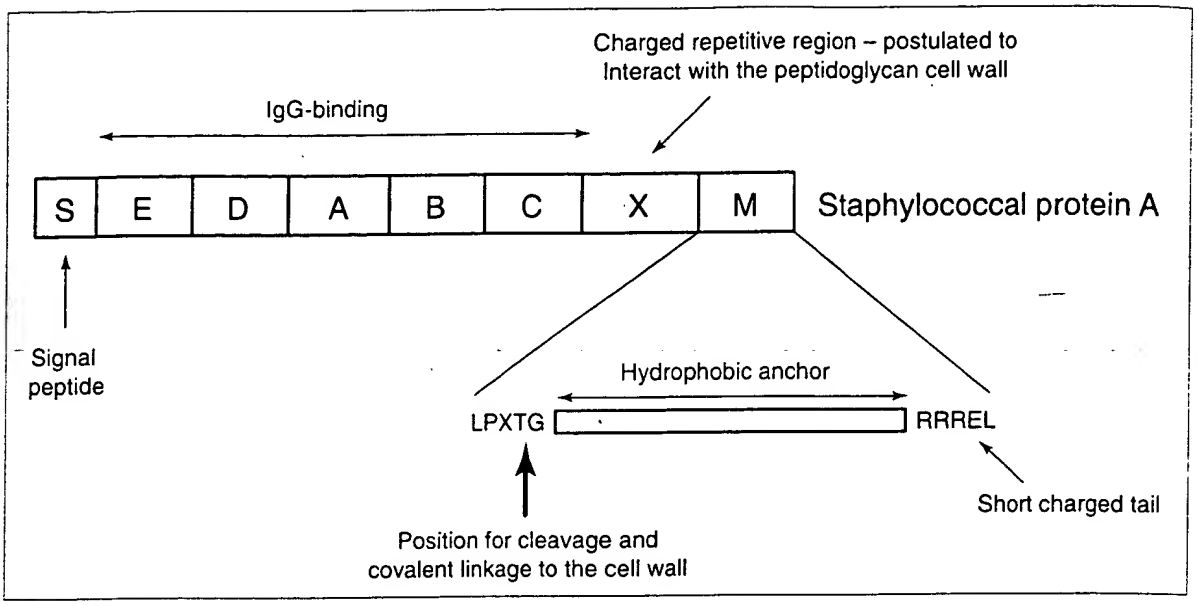

Figure 1

Schematic representation of the different regions of *S. aureus* protein A. The C-terminal regions X and M are used to achieve heterologous surface display. The M region contains the signals for covalent linkage of the chimeric surface protein to the outer bacterial surface[49–52].

protein was expressed on the surface of *Lactobacillus fermentum* and *Lactobacillus sake* (J-C. Piard, pers. commun), indicating the possibility of using lactic acid bacteria as host cells for surface display in the near future. In *Lactococcus lactis*, heterologous proteins have been targeted to the cell membrane by fusion to the C-terminal region of the cell-surface-associated proteinase PrtP (Ref. 42). However, chimeric proteins expressed by this system are not accessible on the outer cell surface[42]. To obtain surface association of heterologous proteins on the *Mycobacterium bovis* strain bacille Calmette-Guérin (BCG), relevant regions from a *Mycobacterium tuberculosis* membrane-associated lipoprotein were fused to the target protein[14]. Recently, heterologous surface expression was achieved in *Bacillus subtilis*[46] by fusion of the target protein to the cell-wall-bound autolysin modifier protein CwbA.

Powerful techniques to monitor and characterize the exposed target proteins are crucial. Traditional techniques for studying the recombinant surface proteins include immunofluorescence and immunogold methods[11] but, although these reveal whether a heterologous protein is produced in an accessible form or not, they fail to give reliable quantitative information about the number of exposed chimeric surface proteins per bacterial cell. Immunofluorescence staining can give misleading results by positive staining of partially lysed cells and should thus be accompanied by additional assays. Flow cytometry, in the form of fluorescence-activated cell sorting (FACS), has been used to characterize surface display on bacteria[14,15,40,41]. However, standard FACS technology does not give direct quantitative information concerning the number of exposed surface proteins. Recently, we (C. Andréoni *et al.*, unpublished) developed a novel technique for quantification of the number of chimeric surface proteins on bacteria. The method uses FACS technology and generates a calibration curve by using nonfluorescent plastic beads, similar in size to bacterial cells, coated with known amounts of monoclonal antibodies. Both the beads and the analysed staphylococcal cells were exposed to a fluorescein isothiocyanate (FITC)-labelled secondary antibody to allow FACS analysis. It was found that recombinant *S. carnosus* cells carried approximately 10^4 surface-displayed antigenic sites per cell (C. Andréoni *et al.*, unpublished).

Vaccine development

Many of the described Gram-negative systems have initially been evaluated in *E. coli* for surface display of various antigenic determinants, and subsequently applied to *Salmonella* spp. These species are of interest for the live, oral delivery of heterologous antigens for immunization[56]. Surface expression in *S. typhimurium* of malarial antigenic determinants using an OmpA system resulted in significant serum antibody responses upon oral immunization of mice[57]. The LamB system has been successfully used for surface display of heterologous epitopes in *E. coli*, producing epitope-specific antibody responses after intraperitoneal immunization of mice with live recombinant bacteria[58]. The system does not, however, seem to be as efficient for surface targeting in *Salmonella*[59,60]. It has been suggested that the *aroA* mutant strains of *Salmonella* frequently used for vaccine delivery are deficient in the translocation of LamB and its derivatives across the cytoplasmic membrane[59]. Immunizations of mice with live *Salmonella dublin* expressing a flagellum with an inserted cholera-toxin[36] or hepatitis-B-virus[35] epitope resulted in antigen-specific antibodies. Furthermore, it was found that in mice partial protection from influenza virus infection could be achieved through

immunization with live *S. dublin* carrying a haemagglutinin epitope from influenza virus in its flagellin[61].

The importance of having the antigenic determinants exposed on the surface of the bacteria when using *Salmonella* for live delivery of subunit vaccines is a subject of debate[62,63]. The initial antigen dose delivered by the recombinant bacteria has been shown to be important in priming the immune system[64], and a 10–100 times increase in the expression level of an antigen expressed in the periplasm is needed to achieve levels of antibodies similar to those induced by the same antigen expressed in a surface-exposed configuration[63]. It has been suggested that epitopes exposed at the bacterial cell surface induce antibody responses in a T-cell-independent manner[58] and that the lipopolysaccharides may serve as an adjuvant.

For the Gram-positive bacteria, research on the use of live bacteria to deliver surface-displayed antigenic determinants has focused on commensal or nonpathogenic bacteria[7,39], although investigations of attenuated pathogenic bacteria, such as mycobacteria[14], have been reported (Table 2). The staphylococcal host–vector systems based on *S. xylosus*[11] and *S. carnosus*[40] can efficiently target heterologous antigenic determinants to the surface of the cells. Both these bacteria are described as nonpathogenic[39,47] and are being used in starter cultures for meat-fermentation applications[47,48]. Both systems have been evaluated for the live bacterial delivery of many immunogens of diverse origin[11,15,38–41]. Oral immunization of mice with live *S. xylosus* carrying a heterologous surface protein comprising a region from streptococcal protein G and a trimerized epitope from respiratory syncytial virus (RSV) elicited serum antibodies to the hybrid surface protein, suggesting display of heterologous epitopes on *S. xylosus* as a potential delivery system for oral vaccination[38]. It has been demonstrated that surface exposure of the antigenic determinants is required with these systems in order to elicit antibody responses[15]. Both systems have proved to be nonpathogenic in mice upon oral or subcutaneous administration[39]. The *S. carnosus* system induced slightly higher serum antibody titres to a surface-displayed model antigen after subcutaneous administration than did the *S. xylosus* system[39]. This could potentially be explained by the higher number of recombinant surface proteins present on the *S. carnosus* cells[41]. *S. carnosus* has the additional advantage of longer persistence (more than 70 h) in the mouse gut[39]. Significant systemic antibody responses were recently reported to surface-exposed model antigens after oral delivery with the *S. carnosus* system[39].

The *S. gordinii* system has also been widely investigated for surface display of heterologous antigens, such as epitopes from papilloma virus and human immunodeficiency virus 1, and a protein allergen from the white-faced hornet[12,43–45]. Oral immunization of mice with the recombinant streptococci has been reported to result in serum IgG responses and significant increases in antigen-specific lung and salivary IgA[12,43–45].

Recombinant BCG carrying a surface-exposed antigen from *Borrelia burgdorferi* was able to evoke protective immunity to *Borrelia* infection in mice[14]. When the same antigen was expressed in either secreted or intracellular form, the corresponding recombinant BCG failed to evoke complete protective immunity[14]. *L. lactis* bacteria expressing the tetanus-toxin fragment C in a membrane-associated (but not surface exposed) configuration were significantly more immunogenic than those encoding secreted or intracellular versions of the same antigen[43]. It can thus be concluded that several of the Gram-positive bacteria are interesting candidates for the delivery of surface displayed heterologous antigens and that the surface association seems to be beneficial.

Other application areas

Surface display of enzymes

Certain enzymes have been expressed with retained activity on the surface of Gram-negative and Gram-positive bacteria (Tables 1 and 2) and the potential use of such recombinant bacteria as novel microbial biocatalysts has been discussed. *E. coli* β-lactamase, which is normally a periplasmic enzyme, has been successfully exposed on the outer surface of *E. coli* using the pullulanase system[32] or the Lpp–OmpA system[17], and the Cex exoglucanase of *Cellulomonas fimi* has been displayed on the surface of *E. coli* using the Lpp–OmpA system[18]. However, not all enzymes can be efficiently exposed on the bacterial surface. Alkaline phosphatase, which is normally periplasmic, was found to be retained in the periplasm of *E. coli* when expressed via the Lpp–OmpA system[65]. The efficacy of using *E. coli* with surface-displayed heterologous enzymes as novel microbial biocatalysts remains to be seen. Surface expression is undoubtedly an inexpensive way to produce immobilized enzymes, but Gram-negative bacteria might suffer from practical drawbacks such as cell lysis. Recently, a lipase from *Staphylococcus hyicus* and *E. coli* β-lactamase were expressed on the outer cell surface of *S. carnosus* with retained activity[19] (Table 2). Approximately 10 000 enzyme molecules were found to be present on each cell[19], and it was suggested that the rigid structure of Gram-positive bacteria would make them particularly appropriate as microbial catalysts[19].

Surface display of antibody fragments and peptide libraries

The expression of functional single-chain antibodies on the surface of *E. coli*[20,21] and staphylococci[22] has opened discussion on whether this strategy would be a way to create inexpensive diagnostic tools or alternatives to the rapidly developing phage technology for the selection of peptides or recombinant antibody fragments from large libraries[4–6]. A random peptide library was recently expressed in a conformationally constrained thioredoxin region, introduced into the flagellin gene of *E. coli* and thus surface-exposed into the *E. coli* flagellum[23]. The epitopes for three different immobilized monoclonal antibodies were mapped by

the selection of bacterial clones in a panning procedure, and the identified consensus epitopes had several amino acids in common with motifs found in the original antigens used to generate the monoclonal antibodies[23]. In addition, active single-chain antibody fragments have been expressed on the surface of *E. coli* using the PAL system[20] and the Lpp–OmpA system[21] (Table 1). A single-chain Fv fragment was recently surface-displayed on *S. carnosus* with retained capacity to bind its antigen (human IgE)[22] (Table 2).

A possible major advantage of the bacterial-display systems over the phage-display techniques lies in the fact that bacterial selection might be accomplished through FACS technology, using FITC-labelled antigens. This would avoid crucial steps in phage-display selection procedures such as immobilization of the antigen, elution of bound phages and reinfection with eluted phages. Filamentous phages are too small to be compatible with current FACS technology[6]. It has, however, been shown that *E. coli* cells expressing functional cell-surface-anchored antibody fragments can be separated by FACS[6,21,36]. Francisco *et al.* performed a key experiment for indicating the feasibility of FACS selection: they mixed control and positive cells at a ratio of 100 000:1 and enriched the antibody-expressing cells to greater than 79% of the final mixture in only three rounds of sorting[6,21].

These results, showing that (1) large protein libraries can be surface displayed on bacteria, (2) scFv fragments can be functionally expressed on bacterial cells, and (3) such cells can be efficiently enriched by FACS technology, should increase optimism for the future use of bacterial display as an alternative or at least a complement to the phage systems.

Environmental applications

A very different application of bacterial surface display was recently suggested by Sousa *et al.*[16], who displayed polyhistidyl peptides on the surface of *E. coli* using the LamB system (Table 1). *E. coli* cells with surface-exposed histidine tags adsorbed approximately 11 times more Cd^{2+} ions than control cells. Such bacteria could perhaps be used for bioadsorption of heavy metal ions, potentially valuable for environmental applications[16].

Concluding remarks and future perspectives

A multitude of heterologous proteins have been targeted and anchored to the cell surfaces of Gram-negative or Gram-positive bacteria, and a number of different application areas have been identified. From a practical point of view, Gram-positive bacteria have certain properties that make them potentially more suitable for bacterial surface-display applications[41]. Firstly, the surface proteins of Gram-positive bacteria seem to be more permissive of the insertion of extended sequences from foreign proteins than the Gram-negative surface proteins[7,22,40,45,54]. For Gram-positive bacteria, most systems for surface display rely on a common mechanism for surface anchoring that allows insertion of heterologous protein regions of several hundred amino acids[7,22,40,45,54]. In contrast, Gram-negative outer membrane proteins, which are surface anchored via multiple passages through the outer membrane, have only the surface loops as 'permissive sites' for the insertion of foreign sequences and normally allow only much shorter insertions (one exception was the demonstration that a 232 amino acid sequence of SpA could be surface exposed on *E. coli* using a permissive site in LamB; Ref. 33). A second, more obvious, advantage with the Gram-positive systems is that translocation through only a single membrane is required to achieve proper surface exposure of the heterologous polypeptide. In the Gram-negative systems both translocation through the cytoplasmic membrane and correct integration into the outer membrane are required for surface display (Fig. 2). Finally, considering the practical handling of the bacteria, Gram-positive bacteria have the additional advantage of being more rigid, due to the thicker cell wall[22] (Fig. 3). However, one potential drawback to the use of Gram-positive bacteria is their lower frequency of transformation compared to Gram-negative bacteria, a factor that is of obvious importance in the creation of large libraries. Nevertheless, transformation frequencies of 10^5 to 10^6 transformants $(\mu g\ DNA)^{-1}$ have been reported for *S. carnosus*[47], which indicates that surface-displayed protein libraries of a significant size could be produced in Gram-positive bacteria. Certain Gram-positive

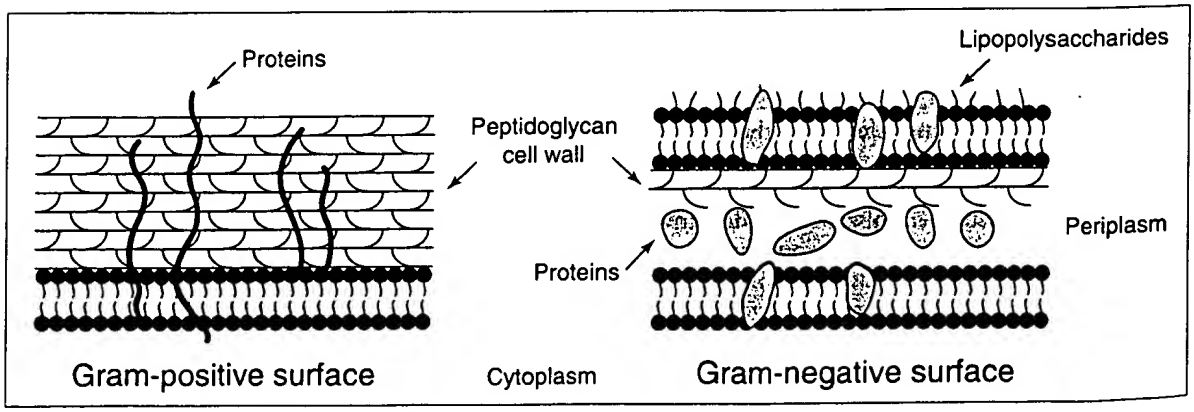

Figure 2
Schematic representation of the differences in the cell surfaces of Gram-positive and Gram-negative bacteria.

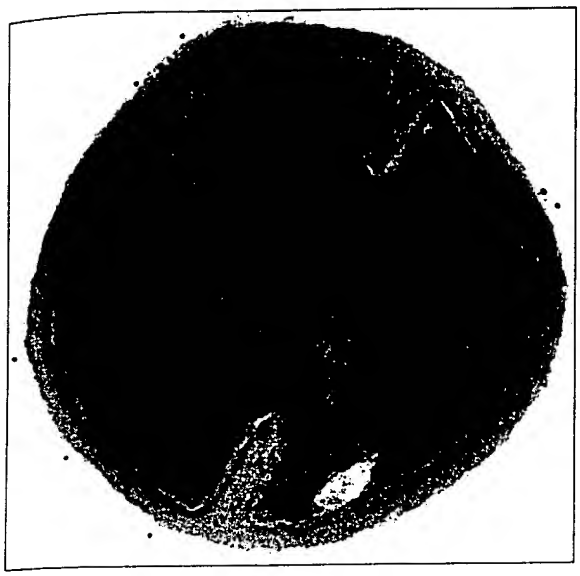

Figure 3

Immunogold electron microscopic picture of an *S. xylosus* cell exposing a streptococcal protein on its surface. The binding of rabbit antiserum specific for the streptococcal protein is detected by the presence of 10 nm diameter colloidal gold particles (*S. aureus*-protein-A–gold conjugate). Note that the cell wall constitutes approximately 10% of the cell radius, which suggests that chimeric surface proteins need to be anchored to the cell wall, and not in the membrane (as for Gram-negative bacteria), to be accessible on the outer cell surface.

bacteria, such as *B. subtilis*, are known to excrete large amounts of proteases, which could cause problems for surface-display applications. In contrast, *S. carnosus* is described as having very low extracellular proteolytic activity[47]. One advantage of the Gram-negative bacteria such as *E. coli* and *Salmonella* is the large number of characterized strains available, which for certain applications have been shown to be useful in the control of surface display[30].

The surface display of active antibody fragments on bacteria could result in a number of new applications. One practical use of this type of recombinant bacteria would be as 'whole-cell monoclonal antibodies' in different diagnostic tests, representing a straightforward and cost-effective way of producing monoclonal antibodies for diagnostic purposes. As a single cell can potentially be detected, it is possible to envisage highly sensitive antibody-based assays. Furthermore, with the help of combinatorial chemistry (such as phage- or bacterial-display technology), there is the possibility of selecting recombinant antibodies or artificial binders with any desired specificity. One interesting application would be the use of combinatorial libraries based on protein domains from bacterial surface proteins (for example, that described by Nord *et al.*[67] utilizing a domain from SpA as a scaffold, which should thus be suitable for display on staphylococcal surfaces).

This concept might also find applications within the field of biofilter development. Ligands selected from combinatorial libraries as specific for a certain compound could be surface displayed on bacteria, which could then be prepared in biofilter form for specific capture. Although enhanced metalloadsorption has been achieved by the surface display of polyhistidyl peptides on *E. coli*[16], the applicability of bacterial biofilter technology remains to be proved.

In the context of vaccine development based on recombinant bacteria carrying heterologous antigenic determinants, the surface display of antibody fragments might become of value. The possibility of expressing recombinant or even 'artificial' antibodies in a functional form on the surface of staphylococci could potentially be used for targeting recombinant bacteria to desired immunoreactive sites. For example, a surface-displayed antibody fragment that binds a certain surface protein or carbohydrate known to be present on an immunoreactive site, such as M cells, could be a means of directing the recombinant bacteria to the desired area and thereby potentially increasing the immune responses to various co-expressed surface antigens.

Among the different bacterial species investigated for vaccine delivery, it is difficult to speculate which types will have the best potential for eliciting protective immunity while being completely safe to use. Food-fermenting bacteria, such as the staphylococcal strains and various kinds of lactic acid bacteria described, as well as commensal bacteria, should be safe to administer orally but may suffer from insufficient immunogenicity. However, the risk of reversion to a virulent phenotype and the potential side-effects in immunocompromised individuals and infants might raise concern over the use of *Salmonella*- or BCG-based recombinant vaccines in humans[68]. One interesting aspect of the choice of bacteria to be used as vaccine-delivery systems is their capacity to withstand the harsh conditions that can be expected during vaccine storage and transportation. In this context, *Bacillus* spores, which are extremely resistant to both heat and cold and which have only recently been considered for vaccine delivery applications[46], may attract future interest.

In conclusion, it is evident that bacterial surface display will be a continuously growing research area in applied microbiology, vaccinology and biotechnology. Both Gram-negative and Gram-positive bacteria of various kinds will be extensively investigated for different biotechnological applications in the near future.

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